Vitreous Humor Biochemical Analysis in Post-mortal Diagnostics of Ruminants

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Introduction

Biochemistry analysis in the live animal is a widely used diagnostic procedure in ruminants.

Having the ability to perform post-mortem biochemistry is an extremely useful tool in determining antemortem biochemical abnormalities and dysfunction, as this provides insight into the significance of the gross pathology, histopathology and ancillary laboratory analyses performed on post-mortal material, especially where gross post-mortem abnormalities are not reported or where a post-mortem is not performed.

Comparison of post-mortal biochemistry to ante-mortal biochemistry on plasma/serum collected from live age-matched herd/flock mates (cohort testing) provides a very powerful herd/flock investigative mechanism.

After death energy metabolism ceases, there is no longer active membrane transport and selective permeability of membranes is lost, resulting in diffusion of solutes. For these reasons, blood samples collected post-mortem are generally of little diagnostic value because of the haemolysis of erythrocytes, the release of cellular breakdown products, and post-mortem bacterial invasion.

This is where the use of vitreous humour (VH) for biochemical /toxicological investigation comes to the fore as the vitreous humour is in the protected environment of the eye.

The presence of the blood-retinal barrier in the eyeball and limited VH vascularization means that the VH is preserved longer post-mortally before degradation compared to blood, urine or other body fluids (thoracic fluid, abdominal fluid, joint fluid, cerebrospinal fluid). Vitreous humour rarely undergoes bacterial contamination. VH is easy to collect and can be of value in disease outbreak investigations up to 48 hours after death and freezing retains VH composition, which allows for prolonged storage prior to testing.

Composition of Vitreous Humour

VH is composed of a gel made up of water, collagen, and hyaluronic acid, admixed with various substances and solutes which are determined by various gradients between plasma and VH with active and passive transport through the blood-retinal barrier (BRB), metabolism in the retina and ciliary body, and diffusion within the vitreous body. The main role of the BRB is the handling of the retina extracellular fluid and VH microenvironment. The BRB structure is a complex unit consisting of the barrier and the ciliary body.

They are fundamental to keeping the eye as a highly controlled site in the body by regulating the contents of its inner fluids; the internal ocular tissues are thus preserved from variations which occur constantly in the whole circulation. There is usually an equilibrium between plasma and VH about various low molecular weight solutes. It is VH moderately stable for a prolonged period after death, which makes it useful for post-mortem analysis.

Magnesium (Mg), Calcium (Ca), Urea, Creatinine, Beta-hydroxybutyrate (BHB), Lactate, Nitrate/nitrite and Ammonia levels remain stable for up to 48 hours post death and freezing maintain levels at pre-freezing concentrations.

Specimen collection, storage and analytical methods

An18G needle is introduced at the scleral margin (corneoscleral junction), directed towards the optic disc, and 2-5 mL of VH aspirated.

Introduction and extraction must be as atraumatic as possible. Gentle VH suction prevents retinal damage that can lead to VH electrolyte rise due to retinal cell disruption or hard aspiration can cause the detachment of retinal fragments making the sample turbid. The VH collagenous gel may plug needles, so, altering the position of the needle and/or degree of aspiration may be required.

If analysis can be performed within 24 hours specimens can be kept and transported at 4ºC, otherwise, samples should be frozen at -20ºC and transported to the laboratory on ice. VH biochemistry is run on standard plasma biochemistry and blood-gas analysers and can be run together with standard blood samples without any recalibrations of equipment required. The only laboratory analytical pre-requisite is that VH is centrifuged at 3000 rpm for 10 minutes prior to analysis to reduce the viscosity of the VH.

Interpretation of Results

Ocular fluid biochemistry should not be used as a diagnostic criterion but more as an adjunct to other diagnostic analyses regarding the animal sampled as well as live age-matched cohort animals. Results must be considered in relation to clinical history, pathology (gross and histopathology), other laboratory analyses on post-mortal specimens, estimated time of death and laboratory analyses of live cohort animals.

<u>Magnesium</u>

Decreased magnesium concentrations in ocular fluids are a reliable indicator of ante-mortal hypomagnesaemia, especially

September 2024

if the sample integrity has been maintained. VH magnesium levels of <0·55 mmol/litre in cattle and <0·65 mmol/litre in sheep are consistent with hypomagnesaemia. If bacterial growth has occurred (VH resistant to post-mortal bacterial overgrowth) or samples have been stored or transported at high temperatures (>37ºC for 48 hours) this can potentially lower VH Mg levels. Normal or elevated levels of magnesium in VH do not exclude hypomagnesaemia, as magnesium contamination of the sample may have occurred as a result of autolysis, contamination with cells or cellular debris (over robust sample collection) or the use of contaminated containers. The correlation of VH Mg concentrations with plasma Mg levels in plasma from herd/flock mates enables a more critical evaluation of the significance of the VH levels.

Calcium

As VH is in equilibrium with ionised calcium, not total plasma calcium, ocular fluids usually have concentrations around 50 % of blood calcium. Low levels of VH calcium (<1 nmol/litre) correlate well to plasma/serum values below normal blood ranges, and are suggestive of terminal Hypocalcaemia. The plasma reference range for calcium in cattle and sheep is 2 to 3 mmol/litre. Such a VH Ca result does not confirm hypocalcaemia, but it increases the probability that hypocalcaemia has occurred prior to death and would indicate that further investigation, such as blood sampling of animals in the cohort, should be carried out to confirm the diagnosis.

Urea and Creatinine

Urea and creatinine provide a reliable indicator of ante-mortal acute and chronic renal failure and levels are sufficiently stable for up to 36 hours after death at ambient temperatures of up to 30°C to provide useful indicators of probable plasma/serum blood urea nitrogen (BUN) and creatinine status. VH post-mortal urea values are on average 0.84x that of plasma/serum levels with creatinine being slightly less representative. However, using plasma/serum reference ranges of cattle, sheep and goats for interpretation of VH levels in the species concerned is considered valid.

Beta-hydroxybutyrate

Beta-hydroxybutyrate (βHB) concentrations in VH correlate well with plasma/serum levels in ruminants and so are a useful indicator of clinical ketosis in cattle and pregnancy toxaemia in small ruminants. Ruminants exposed to "rancid silage" (high in butyric acid) also develop high levels of VH and plasma/serum ßHB. Plasma/serum reference ranges are used for the interpretation of the significance of VH ßHB levels.

D-Lactate and Total Lactate (L+D)

Overproduction of ruminal D-lactate is the cause of acute ruminal acidosis in ruminants and measurement of D-lactate in VH, plasma/serum or rumen fluid is a very specific and reliable assay for the diagnosis of rumen acidosis. The advantage of the VH D-Lactate analysis is that it remains stable in the ocular fluid for at least 4 days and so is not adversely affected by post-mortal decomposition, as analysing rumen fluid pH post-mortally is. The problem with D-lactate analysis is that there are not many laboratories performing the assay. Measuring total lactate is also a reliable indicator of rumen acidosis (although less so than D-lactate) and can be performed on VH via a blood gas analyser. Where increases in lactate are measured in conjunction with decreased blood pH and bicarbonate they provide strong supporting evidence of rumen acidosis. Total lactate exhibits similar stability to D-lactate in ocular fluids.

Nitrate

VH samples are considered the most appropriate sample for the confirmation of nitrate poisoning in ruminants. Post-mortem ocular fluid nitrate concentrations are relatively stable, and although they decline progressively, they continue to be significant for up to 60 hours after death. Post-collection VH has stable nitrate concentration for testing of at least one week if refrigerated at 4ºC and 1 month if frozen at -20ºC. Although VH nitrate fluid levels have been recorded as being 35% lower than those for serum, they are still considered a reliable indicator of poisoning in cattle. The half-life of nitrate is reported at 7.7 hours for cattle and 4.2 hours for small ruminants, therefore, it takes up to 24-36 hours for increased nitrate levels to return to normal levels, if the nitrate source has been removed. Nitrate in serum and ocular fluid was stable in samples stored for 24 hours at 23ºC, 1 week at 4ºC, and 1 month at -20ºC.

Ammonia

Ammonia analysis of VH provides a very useful and reliable indicator of ammonia toxicity (urea poisoning). Samples need to be collected as soon as possible after death (within 4 hours post-mortal in hot ambient temperatures and within 12 hours after death in moderate climates). All specimens for ammonia analysis should be frozen immediately after collection and are only thawed at the time of analysis at the laboratory. Ammonia in vitreous humour increases linearly after death with a wide range in the region of 21-365 umol/L 24 hours after death. In cases of toxicity, however, levels in both cattle and small ruminants are usually well above 1000 umol/L.

Sodium (Na) and Chloride (Cl).

VH sodium (Na) and chloride (Cl) levels are like those in plasma/serum and are stable for 24 hours Na and 48 hours Cl at 24ºC and both significantly longer at 4ºC. Na and Cl analysis in VH in ruminants is used for the investigation of water deprivation/salt poisoning.

Key points for VH Biochemistry Sample Submission.

Collect the VH sample as soon as possible after death into a plain serum tube/container.
Freeze VH sample following collection.
Transport on ice to the laboratory.
Maintenance of the cold chain prior to analysis.
Centrifuge VH prior to analysis.

Reference Ranges:

VH Biochemistry	Bovine	Small Ruminant
Magnesium mmol/L	0.8 – 1.1 mmol/L	0.4 - 0.8 mmol/L
Calcium mmol/L	1.5 – 1.9 mmol/L	1.5 – 2.1 mmol/L
Urea mmol/L	1.0 - 10.0 mmol/L	3.3 – 8 mmol/L
Creatinine umol/L	60 – 190 umol/L	50 – 150 umol/L
Beta-Hydroxybutyrate(BHB) mmol/L	< 0.7 mmol/L	< 0.7 mmol/L
Lactate mmol/L	0.56 – 2.2 mmol/L	1.00 – 1.33 mmol/L
Nitrate mg/L	4.1 mg/L – 5.7 mg/L	4.1 mg/L – 5.7 mg/L
Ammonia umol/L	Normal < 365 umol/L. Toxicity > 1000 umol/L.	Normal < 200 umol/L. Toxicity > 800 umol/L.
Chloride mmol/L	97 – 111 mmol/L	95 – 103 mmol/L

FURTHER READING:

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MULTIPLE-CHOICE QUESTIONS

QUESTION 1

Which of the following body fluids are preserved the longest post-mortally?
a. Cerebrospinal fluid
b. Ocular fluid
c. Joint fluid
d. Urine
e. Thoracic fluid

QUESTION 2

For up to how long after death is vitreous humour suitable to collect for biochemistry analysis?
a. 12 hours
b. 24 hours
c. 48 hours
d. 72 hours
e. 90 hours

QUESTION 3

Which of the following components is not normally found in vitreous humour?
a. Water
b. Collagen
c. Solutes
d. Hyaluronic acid
e. Erythrocytes

QUESTION 4

Which of the following ocular structures maintains the chemical composition of the ocular fluid?
a. Blood retinal barrier
b. Cornea
c. Sclera
d. Retina
e. Choroid

QUESTION 5

How do you reduce the viscosity of vitreous humour prior to laboratory analysis?
a. Collect vitreous humour with an 18 gauge needle
b. Only apply gentle suction when aspirating vitreous humour
c. Repeatedly reposition the needle during sample collection
d. Centrifuge the vitreous humour just prior to analysis
e. Chill the sample post-collection

QUESTION 6

Which of the following factors would have the least influence on the interpretation of vitreous humour biochemistry results?
a. Make and model of the biochemical analyser
b. Clinical history
c. Gross pathology
d. Time of death
e. Laboratory analysis of live cohort animals

QUESTION 7

Which of the following vitreous humour magnesium results would be considered consistent with hypomagnesemia in cattle?
a. 0.65 mmol/L
b. 0.72 mmol/L
c. 0.27 mmol/L
d. 0.59 mmol/L
e. 0.67 mmol/L

QUESTION 8

For how long after death at an ambient temperature of 27°C would urea and creatinine levels in vitreous humour be considered stable?
a. Up to 12 hours
b. Up to 72 hours
c. Up to 24 hours
d. Up to 36 hours
e. Up to 6 hours

QUESTION 9

Which of the following is considered the most appropriate sample for the diagnosis of nitrate poisoning in ruminants?
a. Serum for the measurement of nitrate levels and live animals
b. Vitreous humor collected post-mortally for nitrate analysis
c. Rumen content collected post-mortally for nitrate analysis
d. Implicated feed for quantification of nitrate levels
e. Heart blood collected post-mortally for nitrate analysis

QUESTION 10

Which of the following statements about the submission of vitreous humour samples to the laboratory for analysis is incorrect?
a. Collect the vitreous humour sample into an EDTA blood tube
b. Freeze the vitreous humour sample following the collection
c. Transport the sample on ice to the laboratory
d. Maintenance of the cold chain prior to analysis
e. Centrifuge the vitreous humour prior to analysis

SAVC CPD Accreditation Code:

AC/1833/24

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